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Report

Dose and Time-Course Evaluation of a Redox-Based Estradiol-Chemical Delivery System for the Brain. I. Tissue Distribution

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Brain-enhanced delivery and sustained release of estradiol (E_2) may be potentially useful in the treatments of vasomotor hot flushes and prostatic adenocarcinoma and for fertility regulation. Therefore, we have evaluated a redox-based estradiol-chemical delivery system (E_2-CDS) for the brain. The mechanism of this drug delivery is based on an interconvertible dihydropyridine⇌pyridinium salt redox reaction. In this study, we investigated the dose- and time-dependent effects of E_2-CDS on the tissue distribution of E_2-Q^+ and E_2, the inactive (intermediate) and active metabolites, respectively, of the E_2-CDS. Ovariectomized rats received a single iv injection of E_2-CDS at 0.01, 0.1, or 1.0 mg/kg or an E_2 dose of 0.7 mg/kg or the drug's vehicle, 2-hydroxypropyl-β-cyclodextrin (HPCD), on day 0. Tissue samples including brain and peripheral tissues were then analyzed for both E_2-Q^+ and E_2 at 1, 7, 14, 21, or 28 days following the E_2-CDS administration. Initially, both E_2-Q^+ and E_2 were detected in all tissues analyzed. The dose-distribution and time-course study demonstrates that (1) at 24 hr (1 day) after administration of E_2-CDS, all tissues showed a dose-proportional increase in concentrations of E_2-Q^+ and E_2; (2) the enzymatic oxidation of E_2-CDS to E_2-Q^+ was dose dependent over the 100-fold dose range examined; and (3) the disappearance of E_2-Q^+ as well as E_2 was slow in whole brain and hypothalamus, with an apparent $t_{1/2}$ = 8–9 days, while both of these metabolites were rapidly cleared from plasma, liver, fat, anterior pituitary, kidney, lung, heart, and uterus. Finally, when the kinetic behaviors of E_2-CDS and E_2 were compared on molar basis, the E_2-CDS (1.0-mg/kg dose) produced E_2 concentrations in brain tissue which were 81- and 182-fold greater than those achieved following equimolar E_2 (0.7 mg/kg) injection at 1 and 7 days, respectively. These data demonstrate that the E_2-CDS is much more effective than E_2 itself in delivering the estrogen to the brain. Collectively, these data support the concept of the brain-enhanced delivery and sustained release of E_2 using the redox-based chemical delivery system.

KEY WORDS: chemical delivery system; blood–brain barrier; estradiol; tissue distribution.

INTRODUCTION

Estrogens are intrinsically lipophilic (1) and readily cross the blood–brain barrier (BBB) to gain access to the central nervous system (CNS). However, when inside the CNS, there is no mechanism to prevent their redistribution back to the periphery as blood levels of the steroids decline (2). Indeed, when these hormones are used therapeutically to target the CNS specifically, the steroids tend to equilibrate among all tissues within the body due to their lipophilicity (3). As a result, only a fraction of the administered dose accumulates at or near the site of action in the brain. This property of the estrogens necessitates either frequent dosing of the steroid to maintain therapeutically effective concentrations in the brain or the administration of a depot form of the estrogen (4). Both of these treatment strategies lead to sustained increases in peripheral estrogen levels.

Furthermore, estrogen receptors are present in many tissues including the CNS, where they mediate a myriad of physiological and pharmacological effects (5,6). This further creates the potential of untoward peripheral side effects (7). In fact, constant increases in peripheral tissue exposure to estrogens have been shown in numerous studies to precipitate various peripheral toxicities including risk of breast and endometrial cancer (7,8), cardiovascular morbidity (9,10), and altered metabolism (11).

Since the brain is the primary site where estradiol (E_2) exerts its beneficial effects on the estrogen withdrawal syndromes at the menopause (12), to inhibit gonadotropins secretion for fertility regulation (13,14), to reduce growth of

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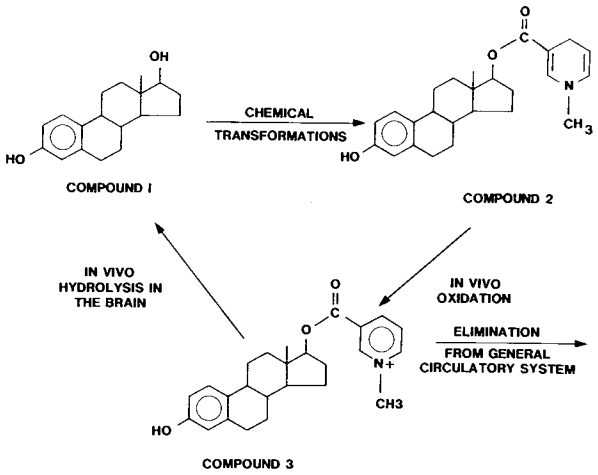

Fig. 1. Schematic representation of the *in vitro* synthesis and *in vivo* transformation of compound 2, the estradiol-chemical delivery system (E_2-CDS). Compound 3 is the quaternary form, E_2-Q^+, of the E_2-CDS, which is locked into the brain but quickly eliminated from the peripheral tissues. Compound 1 is estradiol, released from E_2-Q^+ by nonspecific hydrolysis.

peripheral steroid-dependent tissue tumors such as prostate (15), and to stimulate male and female sexual behaviors (16), a brain-enhanced delivery and sustained release of E_2 are warranted. The ability to deliver E_2 preferentially to the brain, thus sparing non-target site tissues, should improve the therapeutic index of E_2 by (i) increasing the concentrations and/or residence time of E_2 at its receptor site in the brain (ii) and, equally important, decreasing the concentrations and/or residence time of E_2 at the potential peripheral sites of toxicities, thereby decreasing untoward peripheral side effects.

To achieve these therapeutic objectives, a novel estradiol-chemical delivery system (E_2-CDS) for the brain has been developed which offers various potential therapeutic applications (17). The E_2-CDS exploits the unique architecture of the BBB, which normally excludes a variety of pharmacological agents from the CNS as a result of their physicochemical properties (18). The E_2-CDS is a redox-based delivery system and the mechanism of its drug delivery is based on an interconvertible dihydropyridine⇌pyridinium salt carrier (17). Figure 1 shows schematically the structure and the mechanisms leading to brain-enhanced delivery and sustained release of E_2. After administration of the E_2-CDS, the carrier moiety is quickly oxidized to the corresponding quaternary pyridinium ion (E_2-Q^+) in the brain as well as in the systemic circulation. The charged pyridinium–drug complex is thus locked into the brain, while the same moiety rapidly clears from the periphery because of its increased hydrophilicity. Sustained release of the active, parent drug from the charged pyridinium–drug complex occurs in the brain as a result of enzymatic hydrolysis of the ester linkage (17,19).

The present studies were undertaken to determine if the E_2-CDS behaves as predicted on the basis of the physicochemical properties designed into its structure. We determined (1) the effects of E_2-CDS dose on tissue concentrations of E_2-Q^+ and E_2, (2) the effects of E_2-CDS dose on the rate of oxidation of E_2-CDS to E_2-Q^+ and hydrolysis of E_2-Q^+ to E_2, and (3) the effects of E_2-CDS dose on the clearance of E_2-Q^+ and E_2 in a variety of tissues. More specifically, our objectives were to analyze quantitatively both E_2-Q^+ and E_2 (two metabolites of the E_2-CDS) in brain, hypothalamus, anterior pituitary, kidney, lung, heart, liver, fat, uterus, and plasma following a single iv injection of one of several doses of the E_2-CDS over a 28-day time course in ovariectomized (OVX) rats. The pharmacodynamic responses to E_2-CDS administration to the animals used in the present study are reported in the following paper (20).

MATERIALS AND METHODS

Chemical Delivery System

Estradiol-chemical delivery system, E_2-CDS (3-hydroxy-17β-{(1-methyl-1,4-dihydropyridine-3-yl)carbonyl)-

Table I. Effects of Dose on the Oxidation and Hydrolysis of E_2-CDS in a Variety of Tissues *in Vivo*

Tissue	Oxidation (-fold increase)[a]	Hydrolysis[b] at an E_2-CDS dose (mg/kg) of		
		0.01	0.1	1.0
Brain	176	24	23	13
Hypothalamus	112	22	23	19
Anterior pituitary	ND[c]	40	31	ND
Plasma	147	14	4	2
Kidney	103	29	22	27
Lung	116	47	32	24
Heart	125	25	21	29
Liver	73	13	15	6
Fat	77	35	22	7
Uterus	21	31	32	26

[a] Fold increase in E_2-Q^+ concentrations over a 100-fold increase in the E_2-CDS dose.
[b] Average percentage hydrolysis (fraction of E_2-Q^+ hydrolyzed ÷ total E_2-Q^+ × 100) on the first sampling time (day 1).
[c] Not determined due to incorrect extraction of the tissue at day 1.

oxy}-estra-1,3,5-(lO)-triene), and E_2-Q^+ (1-methyl-3-{((3-hydroxyestra-1,3,5-(lO)-triene-17β-yl)oxy)carbonyl}pyridinium iodide) were synthesized as previously reported (17).

E_2-CDS was prepared for injection in aqueous solution containing 20% 2-hydroxypropyl-β-cyclodextrin (HPCD) (w:v).

Animals and Drug Treatment

Adult female Charles River (CD) rats (225-250 g) were purchased from Charles River Breeding Laboratories (Wilmington, MA). Animals were housed in a temperature (23°C)- and light (lights on 0500 to 1900 daily)-controlled room and provided with Purina rat chow and tap water *ad libitum*.

To evaluate the tissue distribution and pharmacodynamics of the E_2-CDS, all animals were bilaterally ovariectomized (OVX) under metofane anesthesia. All experiments were initiated exactly 2 weeks after ovariectomy.

On day 15 after ovariectomy, rats (seven per group per each time point) were administered a single iv injection (tail vein) of the E_2-CDS at doses of 0 (HPCD), 0.01, 0.1, or 1.0 mg/kg body weight or E_2 at a dose of 0.7 mg/kg (equimolar to 1.0 mg of the E_2-CDS).

Tissue and Plasma Collection

Rats (seven per group) were killed by decapitation 1, 7, 14, 21, or 28 days after the drug administration and the trunk blood was collected in heparinized tubes. The blood was centrifuged and the plasma separated and stored at −20°C until hormone analysis. Tissues (whole brain, hypothalamus, anterior pituitary, kidney, lung, heart, liver, fat, and uterus) were dissected immediately following decapitation, rinsed in ice-cold saline, stripped of surrounding connective tissue where necessary, blotted dry on paper, and then stored at −80°C until hormone analysis.

Tissue and Plasma Analysis

Tissue samples of known wet weight at a concentration of 1 mg/20 μl solvent were processed and assayed for E_2-Q^+ and E_2 by the method previously described by us (21,22). Briefly, three steps were used to extract and prepare samples for the radioimmunoassay (RIA) of E_2. The first step was the homogenization and extraction of the biological samples as follows: (a) for E_2 extraction from brain, hypothalamus, anterior pituitary, lung, liver, uterus, and kidney,

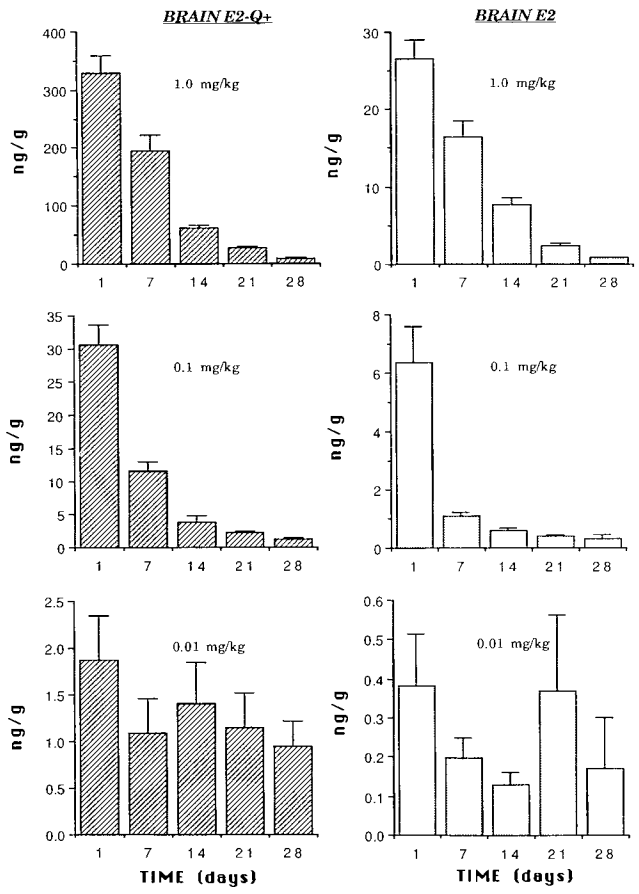

Fig. 2. Dose- and time-dependent effects of the E_2-CDS on E_2-Q^+ concentrations (left panels) and E_2 concentrations (right panels) in whole brain of ovariectomized rats. Animals received a single iv (tail vein) injection of the E_2-CDS on day 0 at a dose of 1.0 mg/kg (upper panels), 0.1 mg/kg (middle panels), or 0.01 mg/kg (lower panels). Represented are means ± SE for n = 7 rats per group per sampling time.

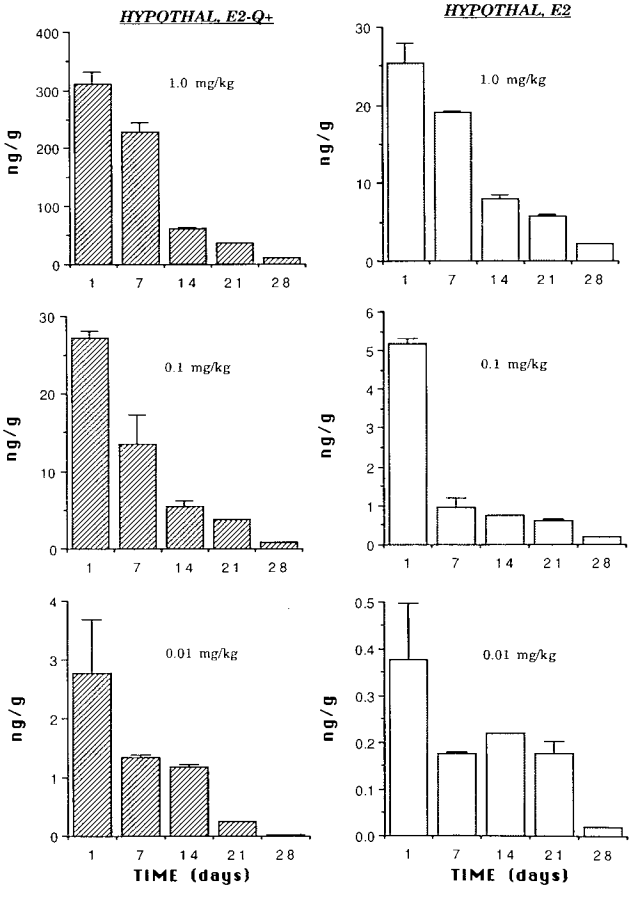

Fig. 3. Dose- and time-dependent effects of the E_2-CDS on E_2-Q^+ concentrations (left panels) and E_2 concentrations (right panels) in hypothalamus of ovariectomized rats. Animals received a single iv (tail vein) injection of the E_2-CDS on day 0 at a dose of 1.0 mg/kg (upper panels), 0.1 mg/kg (middle panels), or 0.01 mg/kg (lower panels). Represented are means ± SE for n = 7 rats per group per sampling time.

100% methanol was used, and for E_2 extraction from fat tissue, 100% acetone was used; and (b) for E_2-Q^+ extraction, all tissue samples were extracted with water/acetone (50:50, v:v). Tissue homogenate pools, at a concentration of 100 mg tissue/2 ml solvent, were prepared for recovery estimation. The second step was the base-catalyzed hydrolysis of the E_2 conjugate (E_2-Q^+) in 1 *N* NaOH solution. The third step utilized solid-phase chromatography with C_{18} reversed-phase columns, which provide a rapid and precise extraction and separation of E_2. The analysis of plasma samples did not require the initial solvent extraction.

Controls (Blanks)

Tissue homogenates or plasma from HPCD-treated rats were analyzed to determine residual E_2 concentrations and thereby served as our estimate of hormone background.

Radioimmunoassays

Coat-A-Count estradiol kits, a solid-phase ^{125}I radioimmunoassay (RIA) designed for the quantitative measurement of E_2 in serum, were purchased from Diagnostic Products Corporation (Los Angeles, CA). Each kit is equipped with human serum-based standards having E_2 values ranging from 20 to 3600 pg/ml (0.07 to 13.2 nmol/liter; technical information from Diagnostic Products).

Cross-reactivity of the E_2 antibody has been determined to be <0.3% for E_2-Q^+ at a concentration of 15 ng/ml and higher (22). Cross-reactivity for estriol and estrone has been reported to be 0.32 and 1.1%, respectively (technical information from Diagnostic Products).

All the purified dried E_2 unknowns were reconstituted in 300 μl of the assay buffer (kit Zero Calibrator) and assayed in duplicate by RIA. The intraassay and interassay coefficients of variation for E_2 were 1.56 and 6.1%, respectively. All samples were determined in 14 RIA runs.

Calculations

Calculated values obtained from the RIA run were adjusted for the volume of the aliquot taken for the RIA, experimental losses during solvent extraction and chromatography, and the weight of the tissue samples used.

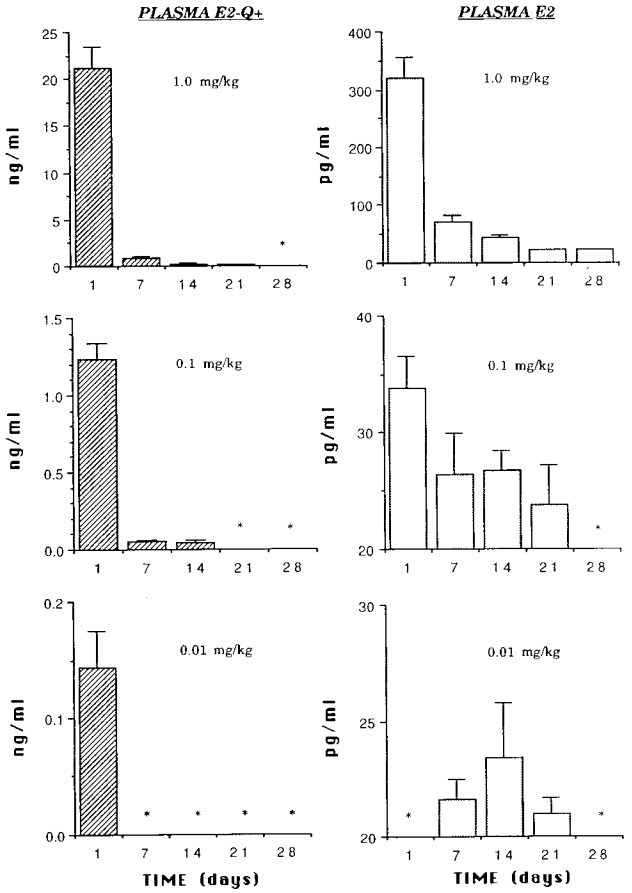

Fig. 4. Dose- and time-dependent effects of the E_2-CDS on E_2-Q^+ concentrations (left panels) and E_2 concentrations (right panels) in plasma of ovariectomized rats. Animals received a single iv (tail vein) injection of the E_2-CDS on day 0 at a dose of 1.0 mg/kg (upper panels), 0.1 mg/kg (middle panels), or 0.01 mg/kg (lower panels). Represented are means ± SE for n = 7 rats per group per sampling time. Asterisks indicate below the sensitivity limit of the assay.

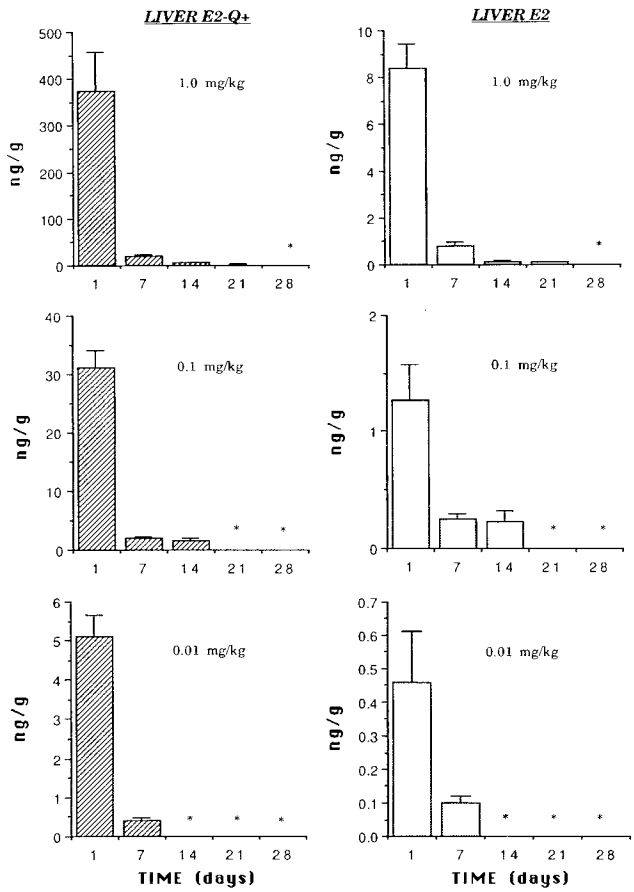

Fig. 5. Dose- and time-dependent effects of the E_2-CDS on E_2-Q^+ concentrations (left panels) and E_2 concentrations (right panels) in liver of ovariectomized rats. Animals received a single iv (tail vein) injection of the E_2-CDS on day 0 at a dose of 1.0 mg/kg (upper panels), 0.1 mg/kg (middle panels), or 0.01 mg/kg (lower panels). Represented are means ± SE for n = 7 rats per group per sampling time. Asterisks indicate below the sensitivity limit of the assay.

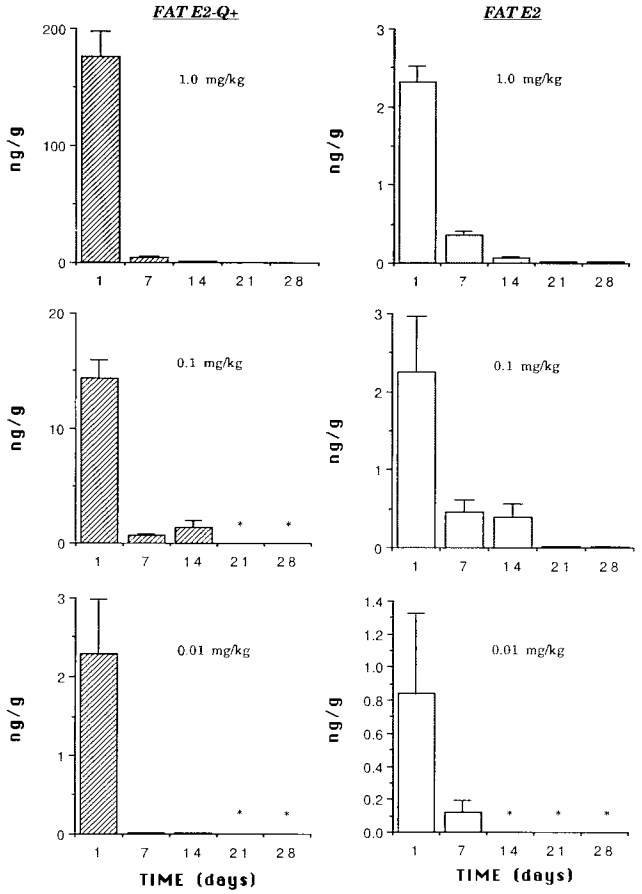

Fig. 6. Dose- and time-dependent effects of the E_2-CDS on E_2-Q^+ concentrations (left panels) and E_2 concentrations (right panels) in fat of ovariectomized rats. Animals received a single iv (tail vein) injection of the E_2-CDS on day 0 at a dose of 1.0 mg/kg (upper panels), 0.1 mg/kg (middle panels), or 0.01 mg/kg (lower panels). Represented are means ± SE for $n = 7$ rats per group per sampling time. Asterisks indicate below the sensitivity limit of the assay.

RESULTS

To estimate the extent of *in vivo* oxidation of E_2-CDS to E_2-Q^+, we determined for each tissue the magnitude of increase in E_2-Q^+ concentrations over the 100-fold increase in E_2-CDS dose administered. The enzymatic oxidation of E_2-CDS to E_2-Q^+ showed a clear dose dependency in brain, hypothalamus, plasma, kidney, lung, heart, liver, and fat tissues (Table I). This dose-related oxidation ranged from 73-fold in liver to 176-fold in whole brain tissue over the 100-fold increase in E_2-CDS dose administered. Uterus was an exception and showed only a 21-fold increase in E_2-Q^+ concentrations over the 100-fold increase in E_2-CDS dose (Table I).

The *in vivo* rate of hydrolysis of E_2-Q^+ to E_2 was estimated for each tissue at each dose of E_2-CDS administered by determining the ratio of E_2 to E_2-Q^+ on the first sampling day (day 1). This ratio remained constant over a 100-fold dose range for the hypothalamus, kidney, heart, and uterus; it decreased moderately (less than 50%) over the 100-fold dose range for the brain, lung, and liver and decreased precipitously for plasma and fat tissue.

At 1 day after administration of E_2-CDS, all tissues showed a dose-dependent increase in concentrations of E_2-Q^+ and E_2. Furthermore, the concentration–time profiles revealed a gradual decline in concentrations of E_2-Q^+ and E_2 in whole brain (Fig. 2) as well as in hypothalamus (Fig. 3), with $t_{1/2}$ = 8–9 days. In contrast, both E_2-Q^+ and E_2 were rapidly cleared from plasma (Fig. 4), liver (Fig. 5), fat (Fig. 6), and anterior pituitary, kidney, lung, heart, and uterus (Tables II and III). By 28 days (the last sampling time) after a single injection of 1.0 mg E_2-CDS/kg, the E_2-Q^+ concentrations remained elevated at 9.8 ± 0.7 ng/g wet tissue (mean ± SE) in brain (Fig. 2, left column, upper panel) and 10.6 ± 0.2 ng/g in hypothalamus (Fig. 3, left column, upper panel). In contrast, peripheral tissues concentrations of E_2-Q^+ were reduced to 2.9 ± 0.8 ng/g in anterior pituitary, 5.2 ± 2.2 ng/g in kidney, 2.9 ± 0.5 ng/g in lung, 1.7 ± 0.3 ng/g in heart, and 2.5 ± 0.7 ng/g in uterus (Table II, % reduction); and E_2-Q^+ values were undetectable in plasma, liver, and fat (Figs. 4–6,

Table II. Effects of the E_2-CDS on the Disappearance of E_2-Q^+ from a Variety of Tissues

Tissue	Dose (mg/kg)	Days after Treatment				
		1[a]	7[b]	14[b]	21[b]	28[b]
Brain	1.0	330	42	82	92	97
Hypothalamus	1.0	311	16	80	88	96
Anterior pituitary	1.0	ND[c]	ND	ND	ND	ND
Plasma	1.0	21	96	99	>99	UD[d]
Kidney	1.0	488	80	96	98	>99
Lung	1.0	717	83	98	99	>99
Heart	1.0	910	82	98	>99	>99
Liver	1.0	375	94	98	>99	UD
Fat	1.0	176	97	99	>99	UD
Uterus	1.0	93	89	93	97	>98

[a] Initial concentration of E_2-Q^+ (ng/g wet tissue) 1 day after administration of the E_2-CDS.
[b] Percentage reduction in E_2-Q^+ concentrations relative to the initial corresponding values.
[c] Not determined due to incorrect extraction of the tissue at day 1.
[d] Undetectable (below the sensitivity of RIA for E_2).

Table III. Effects of the E_2-CDS on the Disappearance of E_2 from a Variety of Tissues

Tissue	Dose (mg/kg)	Days after Treatment				
		1[a]	7[b]	14[b]	21[b]	28[b]
Brain	1.0	27	40	71	91	96
Hypothalamus	1.0	25	24	68	77	91
Anterior pituitary	1.0	ND[c]	ND	ND	ND	ND
Plasma	1.0	0.32	78	87	93	95
Kidney	1.0	135	80	98	>99	UD[d]
Lung	1.0	168	95	99	>99	>99
Heart	1.0	156	82	99	>99	>99
Liver	1.0	8	90	98	>99	UD
Fat	1.0	2	84	97	>99	>99
Uterus	1.0	23	93	94	97	>99

[a] Initial concentrations of E_2 (ng/g wet tissue) 1 day after administration of the E_2-CDS.
[b] Percentage reduction in E_2 concentrations relative to the initial corresponding values.
[c] Not determined due to incorrect extraction of the tissue at day 1.
[d] Undetectable (below the sensitivity of RIA for E_2).

left columns, upper panels) 28 days after administration of a 1.0-mg E_2-CDS/kg dose.

Similarly, E_2 concentrations were maintained relatively high in whole brain (Fig. 2, right panels) and in hypothalamus (Fig. 3, right panels); however, E_2 concentrations in peripheral tissues (except for anterior pituitary and plasma) fell by more than 80% by day 7, and by 97% by day 21, and were undetectable by day 28 (Table III).

In contrast with E_2 concentrations achieved following E_2-CDS administration, E_2 levels in tissues following equimolar E_2 administration were remarkably low (Table IV), and in all tissues examined, the clearance of E_2 was rapid (Table IV). A comparison of the tissue levels of E_2 achieved at day 1 revealed that following E_2-CDS administration, brain (Fig. 2) and hypothalamus (Fig. 3) levels of E_2 were 88- and 22-fold greater, respectively, than levels observed in these tissues following E_2 administration (Table IV). By 7 days after treatment, the E_2-CDS produced brain and hypothalamic E_2 concentrations that were 182- and 55-fold greater, respectively, than those achieved by an equimolar E_2 dose.

DISCUSSION

This detailed dose-distribution and time-course study demonstrates that (i) the enzymatic oxidation of E_2-CDS to E_2-Q^+ is dose dependent, and with the possible exception of the uterus, the oxidation is not saturable over the 100-fold dose range tested; (ii) the hydrolysis of E_2-Q^+ to E_2 was dependent upon the tissue analyzed and appeared to be saturable only in plasma and fat and, to a lesser extent, in brain, lung, and liver; and (iii) the disappearance of both E_2-Q^+ and E_2 was slow in brain tissue and rapid in all peripheral tissue tested. Collectively, these data are consistent with the expected behavior of the E_2-CDS (17).

Dose and time-course profiles revealed that E_2-Q^+ persists in brain tissue as well as in hypothalamus, with a $t_{1/2}$ = 8–9 days, but it is rapidly cleared from the periphery. We have previously shown, in intact male rats, a similar half-life for E_2-Q^+ in brain tissue (19). These estimates of the half-life of E_2-Q^+ in brain are in accordance with reports which utilized different analytical techniques and E_2-CDS doses (23). This clearance of E_2-CDS appears to be independent of dose since similar values have been obtained in studies using doses of E_2-CDS ranging from 0.01 mg/kg (present report) to 15 mg/kg (23). Further, this long half-life of E_2-Q^+ in brain tissue does not appear to be an artifact of its sustained production from E_2-CDS since the half-life of the delivery system itself in brain tissue is only 29.2 min, indicating rapid oxidation to E_2-Q^+ (17,24). Thus, as predicted previously by us (17), the unique features of the BBB appear to contribute to the chronic retention by the brain of the charged, hydrophilic E_2-Q^+.

The anterior pituitary exhibited slower elimination of the metabolites of E_2-CDS (E_2-Q^+ and E_2) than other peripheral tissues. By 7 days following administration of a 1.0-mg E_2-CDS dose, the hypothalamic–anterior pituitary E_2-Q^+ ratio was 1.3 and then increased to about 4-fold by 28 days. Similarly, the hypothalamic–anterior pituitary E_2 ratio

Table IV. Effects of an Equimolar Dose of E_2 (0.7 mg/kg) on the Tissue Concentrations of E_2[a]

Tissue	HPCD Vehicle[b]	Days after Treatment	
		1[c]	7[c]
Brain	0.05 ± 0.03	0.33 ± 0.04	0.09 ± 0.07
Hypothalamus	0.03 ± 0.01	1.16 ± 0.14	0.35 ± 0.08
Anterior pituitary	0.02 ± 0.01	0.99 ± 0.07	0.28 ± 0.02
Plasma	UD[d]	0.03 ± 0.01	0.02 ± 0.01
Kidney	UD	0.55 ± 0.13	0.04 ± 0.01
Lung	UD	0.26 ± 0.30	0.01 ± 0.01
Heart	UD	0.14 ± 0.04	0.01 ± 0.01
Liver	0.02 ± 0.01	0.80 ± 0.20	0.11 ± 0.04
Fat	0.05 ± 0.03	1.12 ± 0.29	0.11 ± 0.05
Uterus	UD	2.08 ± 0.65	0.17 ± 0.14

[a] This dose of E_2 (0.7 mg/kg) is equimolar to 1.0 mg E_2-CDS/kg dose.
[b] Residual E_2 concentrations (ng/g wet tissue; mean ± SE).
[c] E_2 concentrations (ng/g wet tissue; mean ± SE) following administration of E_2.
[d] Undetectable (below the sensitivity of RIA for E_2).

was 1.2 on day 7 and this ratio was maintained throughout the 28-day time course. This relative persistency of both E_2-Q^+ and E_2 in the anterior pituitary may be caused by the anatomical relationship between the hypothalamus and the pituitary gland. Estradiol released upon the hydrolysis of E_2-Q^+, or the E_2-Q^+ itself, which is locked into brain, could be delivered directly to the pituitary by the capillary plexus of the hypophyseal portal system. These capillaries in the median eminence lack features of other brain capillaries and hence are not part of the BBB (25). Thus, the median eminence would not be expected to prevent the efflux of E_2-Q^+ from brain, and transfer of E_2-Q^+ to anterior pituitary can be expected.

Plasma also showed, after day 1, a residual but detectable E_2 concentration throughout the time course of the 1.0-mg E_2-CDS dose and through the 21-day time course of the 0.1-mg dose. This prolonged and residual E_2 in plasma is likely to be a result of a continuous redistribution of E_2 liberated from E_2-Q^+ in the brain or other tissue down its concentration gradient into the general circulation.

The oxidation of E_2-CDS to E_2-Q^+ in uterine tissue does not appear to be dose dependent, since a 100-fold increase in dose resulted in a 21-fold increase in E_2-Q^+ concentration. This observation is in part an artifact of the observed uterine hypertrophy in animals treated with the E_2-CDS (26). At day 1 after E_2-CDS treatment, a 100-fold increase in the E_2-CDS dose results in a 65% increase in uterine weight, thus reducing the values for E_2-Q^+ when normalized for tissue weight. Additionally, in OVX rats, blood flow to the uterus is low, and thus the expected rapid oxidation of E_2-CDS to E_2-Q^+ would be less likely in that tissue. Finally, we cannot rule out the possibility that in the uterus of OVX rats, the enzymatic oxidation of E_2-CDS to E_2-Q^+ is a saturable process and is thereby independent of the dose of E_2-CDS administered.

To demonstrate further the preferential deposition and retention of estrogen in the CNS with the E_2-CDS, one dose of E_2 (equimolar to 1.0 mg E_2-CDS dose) was also studied. As shown in Table IV, E_2 concentrations in the CNS tissues of rats treated with E_2 were slightly increased on day 1 and were just above the detection limits of the assay at 7 days. In contrast, the 1.0-mg E_2-CDS dose resulted in brain E_2 concentrations that were 81- and 182-fold greater than those achieved following E_2 injection at 1 and 7 days, respectively. These data demonstrate that the E_2-CDS is much more effective than E_2 itself in delivering and retaining the estrogen in the brain.

Collectively, these observations are consistent with the proposal that E_2 can be preferentially delivered to the brain using a redox-based chemical delivery system, an inert molecule which requires several steps in its conversion to the parent drug (18,27,28). The multiple, facile conversions including oxidation and hydrolytic cleavage not only may lead to preferential E_2 delivery and sustained release/effects but also may act to decrease the toxicity of the drug. A preferential and sustained CNS estrogen delivery can be potentially useful since estrogens are known to influence a variety of CNS actions (29,30).

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